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Changes in the Antioxidant Content of Moringa Leaves (*Moringa oleifera*) Fermented with *Trichoderma koningiopsis* AA1

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Moringa oleifera leaves are known as a source of antioxidants. To further enhance the role of antioxidant nutrients in moringa leaves as livestock feed, an effective and inexpensive method is fermentation treatment. This study aims to determine changes in antioxidant content in fermented moringa leaf flour using *Trichoderma koningiopsis* AA1. The fermentation experiment was designed with a Completely Randomized Design. The research treatments included fermentation periods of 0, 4, and 8 days with three replications. The fermented moringa leaf flour was then analyzed for its antioxidant content, including flavonoids, vitamin E, β-carotene, and total phenols. The results showed contents of flavonoids, vitamin E, and β-carotene increased content from day 0 to day 4 and increased further from day 4 to day 8. The total phenol content decreased from day 0 to day 4 and reduced from day 4 to day 8. The study concluded that fermentation of moringa leaf flour using *Trichoderma koningiopsis* AA1 was proven to have a significant effect ($P<0.05$) on increasing flavonoid, vitamin E, and β-carotene content and reducing total phenol content. The best fermentation time to increase antioxidant content occurred on the 8th day of fermentation.

Keywords: Bioactive compounds, antibiotic growth promotors, phenols, *Aspergillus niger*, aflatoxins, silage feed.

INTRODUCTION

Research on *Moringa oleifera* continues to grow along with the increasing need for natural ingredients that are rich in nutrients and antioxidants (Mehlomakulu & Emmambux, 2022). Dried Moringa leaves contain 28.4% protein, 2.7% fat, 57% carbohydrates, 12.6% fiber, and 1600-2200 mg calcium (Hanif & Berawi, 2022). Moringa leaves are rich in protein and contain a variety of bioactive compounds with antioxidant properties (Segwatibe et al., 2023). The provision of moringa leaf supplements can also replace antibiotic growth promoters (AGPs) as a feed additive in poultry diets (Setiadi et al., 2023). Although classified as highly nutritious animal feed, Moringa leaves have a deficiency in the form of antinutrient content in the form of total phenols which can inhibit growth in livestock (Lestari et al., 2021). Cooking and fermentation can reduce the amount of anti-nutrients in green fodder, such as moringa leaves.

Fermentation is the most effective method in processing green fodder to increase the availability of nutrient materials and reduce antinutrients in green fodder with the help of microorganisms. The fermentation process relies on the biochemical functions of the fungus *Trichoderma koningiopsis* AA1 to break down organic matter in aerobic or semi-aerobic environments (Dionisi, 2021; Chai et al., 2022). Microorganisms in the fermentation process increase the availability of nutrients by degrading complex nutrient structures into simpler ones (Sakandar et al., 2023). The fermentation process can enhance the levels of bioactive compounds (Yang et al., 2024). Fermentation can enhance the bioactive compound content of the ingredients (Ansory et al., 2023). The fermentation process can also change the chemical composition of the material by increasing the biochemical and physiological activity of organic compounds (Tran et al., 2023). Fermentation using *Trichoderma sp*. fungi can degrade antinutrients in materials and improve their nutritional quality (Mulyono et al., 2024). As a decomposer, the fungus

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Trichoderma sp. produces enzymes capable of breaking down fiber components (Mulyono et al., 2025). Previous research used *Aspergillus niger* sp. fungus to ferment moringa leaves to increase the availability of nutrients from moringa leaves (Fenita et al., 2024). *Aspergillus niger sp.* and *Trichoderma sp*. have similarities in decomposing organic compounds. However, some species of *Aspergillus niger sp*. produce aflatoxins. Previous studies have shown that aflatoxin produced by *Aspergillus niger* sp. can cause health problems in livestock (Lisu et al., 2023). Fermentation of moringa leaf flour with *Trichoderma sp*. microorganisms is believed to provide better results than *Aspergillus niger sp*. in increasing nutrient bioavailability, increasing moringa leaf flour's antioxidant value and its safer application. Previous studies have examined the nutritional content of materials fermented with Trichoderma sp. but have not specifically discussed the increase in antioxidant value, especially in Moringa leaves (Mulyono et al., 2021).

The antioxidant content in Moringa leaves has many vital roles, including reducing oxidative stress (Priyanto et al., 2023; Al-Muhe et al., 2024). Increased oxidative stress can cause damage to cells in the body (Salami et al., 2016; Frasiska & Kusmayadi, 2020). Previous research has demonstrated that antioxidant supplementation can mitigate the adverse effects of oxidative stress (Ngadiman et al., 2023). The antioxidant content in moringa leaf flour can increase the growth of body cells damaged by oxidative stress (Lee et al., 2017; William et al., 2023). The compounds in Moringa leaves that function as a source of antioxidants are flavonoids (Kurang et al., 2020), vitamin E (Mubarak et al., 2017), and β-carotan.

This study analyzed changes in the content of antioxidant compounds in Moringa leaf flour fermented with *Trichoderma koningiopsis* AA1. This study is expected to contribute to developing potential antioxidant sources from local materials and obtain comprehensive data on the antioxidant content value of moringa leaf flour fermented using *Trichoderma koningiopsis* AA1 fungi with different fermentation periods.

MATERIALS AND METHODS

***Substrate preparation*:** The substrate used in this study was moringa leaves that were dried in the sun for 4 days. The dried moringa leaves were blended until they became flour and then sieved with a 1mm sieve. Moringa leaf flour weighed as much as 1000 g and sterilized with an autoclave at 15 psi for 15 minutes. The sterilized moringa leaf flour is ready to use.

***Microbial preparation*:** Microbes were prepared by making a molasses solution by adding 300 ml of distilled water and 20 ml of molasses into a 1000 ml Erlenmeyer flask. The molasses solution was sterilized with an autoclave at 15 psi for 15 minutes. Starter *Trichoderma koningiopsis* AA1 (STk-AA1) in the form of flour. A total of 20g was put into an Erlenmeyer flask containing molasses solution, then closed and incubated aerobically for 24 hours (Mulyono et al., 2025).

***Experimental design and data analysis*:** The experiment used a completely randomized design (CRD) with one-way patterns and different fermentation treatments of 0 days (control), 4 days, and 8 days. Each treatment was repeated 3 times. The observation variable data were analyzed using the One-Way Analysis of Variance (ANOVA) and continued with the Duncan test to see significant differences between treatments.

***Fermentation procedure*:** The fermentation process was done by inserting 1000 g of moringa leaf flour, 300 ml of microbes, and 500 ml of sterile distilled water into a plastic container. All ingredients were stirred until evenly mixed. The mixture of substrate and microbes was inserted into a 42 cm bioreactor made of bamboo with a substrate mixture thickness of 2 cm, then closed and placed on a stacking rack, incubated for a period according to the treatment. Fermented moringa leaf flour was harvested by drying it using an oven at a temperature of 70° C for 2 days. Fermented Moringa leaves are mashed with a blender, packed with a vacuum sealer, and labeled.

***Flavonoid content analysis*:** Measurement of flavonoid content using the Spectrophotometry method (Hasan et al., 2023). Flavonoid content was measured by weighing a sample of fermented *Moringa oleifera* leaf flour as much as 5 g into a 100 ml Erlenmeyer flask. Using a measuring flask, ethanol 96% was added until it reached the calibration mark. The solution was filtered using filter paper; the clear filtrate was taken as much as 1 ml and put into a test tube. $AlCl_3$ 5% solution as much as 2 ml and ethanol 96% as much as 7 ml were added to the test tube and shaken using a vortex until evenly mixed. The absorbance of the solution was measured using a spectrophotometer at a wavelength of 415 nm. The absorbance was plotted on the Quercetin standard curve.

***Vitamin E analysis as total tocopherol*:** Vitamin E content will be analyzed using the analysis of vitamin E as total tocopherol AOAC 1988, as done (Wulan, 2024). The procedure for analyzing vitamin E as total tocopherol is carried out by weighing 1 g of sample, then dissolving it in 10 ml of ethanol. A sample of the solution is taken as much as 1 ml, and 3.5 ml of 2.2 Bipyridine 0.07% solution is added, followed by 0.5 ml of $FeCl_3$ 0.02%. The solution is then diluted to a volume of 10 ml using 96% ethanol. The absorbance is measured at a wavelength of 520 nm. The vitamin E content is determined by plotting the absorbance of the sample solution on the standard curve regression equation.

***Analysis of β-Carotene content*:** The β-Carotene method is analyzed using the spectrophotometric content (Umar et al., 2022). Analysis was done by weighing 5 g of the sample and grinding it with petroleum ether (1:1) and carotene-free sand. The extract was filtered, separated with distilled water, and the upper layer containing carotene was separated. Anhydrous Na_2SO_3 was added to remove water before being diluted with

petroleum ether, and the absorbance was measured at 450 nm. The β-carotene filtrate was loaded into a chromatography column containing glass wool, Al_2O_3, anhydrous Na_2SO_3, and cotton, then activated at 180°C for 2 hours. The β-carotene fraction was collected, the column was washed with petroleum ether and acetone (10:1), then the solution was diluted with petroleum ether. The absorbance was measured at 450 nm. The absorbance of the sample solution was plotted on a standard curve.

***Total phenol measurement using the Folin-Ciocalteu method*:** Total phenol measurement was performed with the results expressed in gallic acid equivalents (GAE) (Rafsanjani et al., 2024). Total phenol measurement using the Folin-Ciocalteu method was carried out by inserting 0.5 mL of standard solution into a test tube, adding 2.5 mL of diluted Folin-Ciocalteu reagent. After 5 minutes, 2 mL of 7% sodium carbonate was added, incubated for 30 minutes at 40°C. The absorbance was measured at a wavelength of 750 nm. The absorbance of this sample solution was plotted on a standard curve.

RESULTS AND DISCUSSION

Antioxidants in *Moringa oleifera* leaf flour can protect cells from damage caused by free radicals that cause various degenerative diseases. Natural sources of antioxidants such as polyphenols and flavonoids are abundant in plants and can be increased by fermentation. The fermentation process with the microorganism *Trichoderma koningiopsis* AA1 (STk-AA1) helps release antioxidant compounds and stimulates the production of secondary metabolites that increase antioxidant activity. The results of measuring the research variables of fermented moringa leaf flour with STk-AA1 in the treatments of 0 days, 4 days, and 8 days showed a significant increase in the content of flavonoids, vitamin E, and B-carotene and showed a significant decrease in the total phenol content; the measurement results can be seen in (Table 1).

Table 1. Average results of antioxidant measurement of moringa leaf flour fermented using *Trichoderma koningiopsis* AA1.

Variables	Fermentation Time		
	0 Day	4 Day	8 Day
Flavonoid (%)	0.59[a]	0.74[b]	0.97[c]
Vitamin E (mg/100g)	163.72[a]	196.45[b]	261.09[c]
β-Karoten (mg/100g)	1.15[a]	1.59[b]	1.80[c]
Total Phenol (%).	1.96[c]	1.57[b]	1.33[a]

[abc] = on the same line shows a significant difference (P ≤ 0.05)

***Flavonoid content changes in moringa leaf flour fermented using Trichoderma koningiopsis AA1*:** Flavonoids are secondary metabolites known as potent antioxidants beneficial for livestock health. In animal husbandry, flavonoids are essential in increasing livestock immunity, reducing oxidative stress, and improving feed efficiency and productivity. One potential natural source of flavonoids is moringa leaves, which are rich in bioactive compounds. Efforts to increase flavonoid content in moringa leaves can be made by fermentation, one of which is by using STk-AA1.

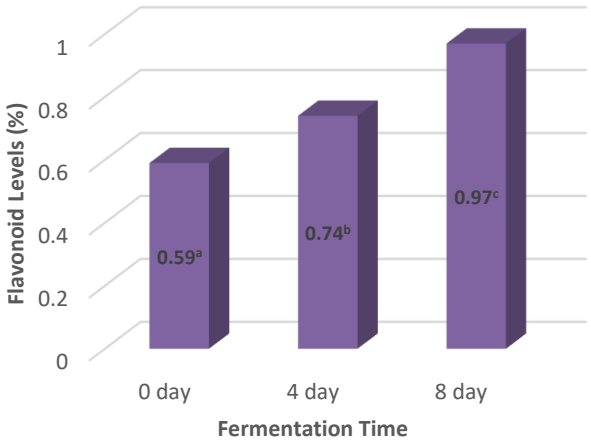

Figure 1. Flavonoid content of moringa leaf flour fermented using *Trichoderma koningiopsis* AA1.

Fermentation using STk-AA1 has been proven to increase flavonoid levels (Table 1) significantly, so it has the potential to be an innovation in providing higher quality and high functional value animal feed. The results of the analysis using variance (ANOVA) showed a very significant increase ($P < 0.05$) in flavonoid content as the fermentation time increased. The graphical response to the rise in flavonoid content in Moringa leaf flour fermented using STk-AA1 can be seen in Figure 1.

This significant increase indicates that biosynthesis occurs in *Moringa oleifera* leaf flour fermentation with STk-AA1 in producing flavonoid compounds. Fermentation of Moringa leaf flour with STk-AA1 is effective in increasing flavonoid content through the mechanism of plant cell wall degradation because it can produce fibrinolytic and hydrolytic enzymes such as cellulase, hemicellulase and pectin, which can decompose the main components of plant cell walls in the form of cellulose, hemicellulose, and pectin (Iannaccone et al., 2022; Li et al., 2022). The degradation process triggers the release of phenylpropanoid precursors, such as p-coumaric acid and cinnamic acid, which play a role in flavonoid biosynthesis through phenylalanine metabolism. Cell wall degradation allows these precursors to enter secondary metabolic pathways, thereby increasing flavonoid production. This process begins when the enzyme PAL converts phenylalanine to p-coumaric acid, then 4CL converts it to p-coumaric-CoA. Next, CHS converts p-coumaric-CoA to chalcones, which are then converted to flavanones such as naringenin by CHI. Naringenin can be converted to flavonols

such as quercetin and kaempferol by FLS or to anthocyanins through other pathways (Dong & Lin, 2021).

Flavonoids have many benefits in animal husbandry, especially as antioxidants that can improve livestock health and maintain feed quality. Flavonoids have antimicrobial properties that can inhibit the growth of parasites, bacteria, and fungi so that they can support the health of the livestock's digestive tract (Saelan & Nurdin, 2019). Feed ingredients rich in flavonoids can help maintain the quality of animal feed during storage by preventing fat oxidation and inhibiting the growth of pathogenic microorganisms (Kalogianni et al., 2020). Flavonoid compounds can improve the immune system of livestock, making them more resistant to infections caused by bacteria and viruses (Chagas et al., 2022). Flavonoids as natural feed additives that can replace synthetic antibiotics in animal feed (Wang et al., 2024). In addition, flavonoids can improve feed efficiency (Tchana et al., 2025), improve growth and meat quality (Zhang & Kim, 2020), improve milk quality (Ponnampalam et al., 2022), and improve egg quality (Vlaicu & Untea, 2024). The flavonoid content in Moringa leaves fermented with STk-AA1 increases the potential for using antioxidant sources from local materials in the livestock industry, which is healthier and more environmentally friendly.

The flavonoid content in fermented moringa leaf flour using STk-AA1 continued to increase until the 8th day, indicating that a longer fermentation process can increase the number of flavonoids. Further research is needed on the optimal limit of moringa leaf fermentation using STk-AA1 to determine the best time for fermentation using STk-AA1 without damaging bioactive compounds due to the fermentation process being too long. Research is also needed on animal feed to determine the effects of giving fermented moringa leaf flour with STk-AA1 microorganisms.

***Changes in vitamin E content of moringa leaf flour fermented using Trichoderma koningiopsis AA1*:** Vitamin E is one of the essential nutrients in animal feed that plays a role in maintaining health, improving the immune system, and supporting livestock growth and reproduction. Vitamin E is an antioxidant that helps protect cells from damage due to oxidative stress that can affect livestock performance and immunity. Providing the correct vitamin E can help increase livestock productivity. One natural source of vitamin E is moringa leaf flour, which is rich in nutrients but has limited bioavailability when given fresh. Proper processing can increase the availability of vitamin E and improve its digestibility. A practical method in processing moringa leaf flour-based feed (*Moringa Oleifera*) is fermentation. Using *Trichoderma koningiopsis* AA1 (STk-AA1) Starter in fermentation can increase the vitamin E content in moringa leaf flour. This process can break down complex compounds, synthesize additional vitamins, and help release nutrients.

The results of the study of fermentation of moringa leaf flour with STk-AA1 were proven to increase the Vitamin E content in moringa leaf flour (Table 1). The Vitamin E content increased significantly ($P<0.05$) based on the results of the analysis of variance (ANOVA). This shows that the difference in fermentation time with STk-AA1 significantly increases the Vitamin E content in moringa leaf flour. The graphic response of the increase in Vitamin E content in moringa leaf flour fermented using STk-AA1 can be seen in Figure 2.

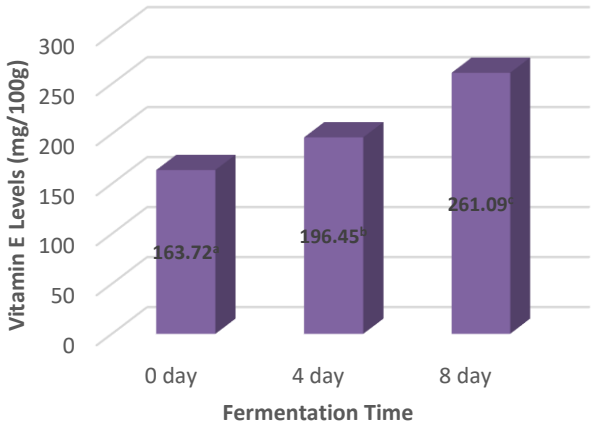

Figure 2. Vitamin E Content of Moringa Leaf Flour Fermented Using *Trichoderma koningiopsis* AA1.

The increase can occur because STk-AA1 produces hydrolytic enzymes in the form of cellulase and hemicellulase enzymes, which function to degrade plant cell walls (Novy et al., 2021). The degradation process releases bound nutrients as vitamin E precursors, such as tocopherols and tocotrienols (Gabriele & Pucci et al., 2022). Fermentation can also synthesize bioactive compounds that increase the levels of vitamin E in the ingredients (Zhao et al., 2021). This process increases the availability of vitamin E from moringa leaf flour.

Vitamin E plays a vital role in maintaining health and increasing livestock productivity. Vitamin E, as an antioxidant, can protect cells from damage caused by free radicals and increase livestock resistance to infection and disease (Xiao et al., 2021). Vitamin E plays a role in muscle formation, increasing feed efficiency and improving meat production (Yaremchuk et al., 2022). Vitamin E compounds can prevent fat oxidation, thereby improving the quality and shelf life of meat (Yaremchuk et al., 2022). In addition to increasing meat production, vitamin E can also increase reproductive productivity. Previous studies have shown that vitamin E plays a role in improving embryo health during pregnancy in cows (Maddahi et al., 2024). Increased productivity also occurs in sheep, where adding vitamin E can increase the potential for multiple births (Ataman et al., 2023). Vitamin E can also help farmers overcome stress due to environmental changes, transportation, and extreme

weather conditions (Zhang et al., 2022). Vitamin E deficiency in livestock can cause various health problems in the form of white muscle disease in cows and goats (Yogeshpriya et al., 2025) and influence increased productivity in poultry (Fu et al., 2022). The potential of vitamin E from local ingredients is an alternative to fulfilling more affordable nutrition. By providing sufficient vitamin E, livestock become healthier, overall productivity increases, and immunity can be maintained. The vitamin E content in moringa leaf flour fermented with STk-AA1 continues to increase until the 8th day; this indicates that a longer fermentation time will increase the vitamin E content. However, further research is needed to determine the optimal fermentation time limit to not damage the Vitamin E content due to degradation. Research is also required on the effects of giving moringa leaf flour fermented using STk-AA1 to livestock.

***Changes in β-Carotene levels of moringa leaf flour fermented using Trichoderma koningiopsis AA1*:** β-Carotene is a natural pigment that is classified as a carotenoid compound and acts as a precursor to vitamin A (Ebadi et al., 2023). This compound is essential in livestock health, reproduction, and product quality. β-carotene in animal feed comes from natural ingredients rich in carotenoid pigments. One of the greens that is high in β-carotene is Moringa leaves (*Moringa oleifera*). The low absorption value of β-carotene in the body of livestock requires further processing. One processing method that can increase β-carotene content is fermentation using *Trichoderma koningiopsis* AA1 (STk-AA1). Analysis of variance (ANOVA) showed that the β-carotene content of fermented moringa leaf flour with STk-AA1 increased significantly with increasing fermentation time (Table 1). This indicates that the fermentation process has a significant effect ($P < 0.05$) on increasing β-carotene levels in moringa leaf flour. The graphic response of the increase in β-carotene content in fermented moringa leaf flour using STk-AA1 can be seen in Figure 3

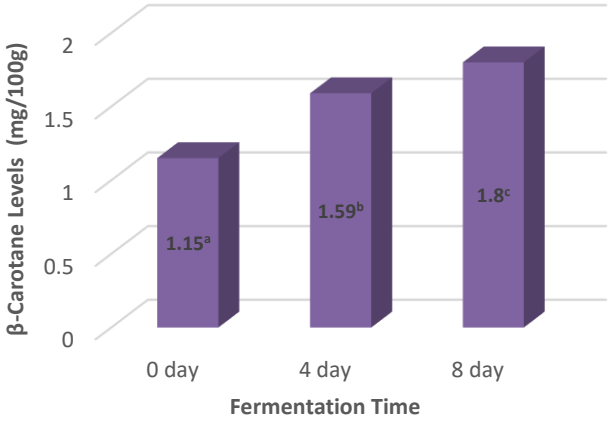

Figure 3. β-Carotene content of moringa leaf flour fermented Using *Trichoderma koningiopsis* AA1.

The increase in β-Carotene levels in Moringa leaf flour during fermentation is caused by the primary mechanism of cell structure breakdown by STk-AA1 by producing enzymes that degrade cell walls. The broken molecules cause β-Carotene to be released more easily, increasing its availability in free form (Bagy et al., 2016). Stimulation of metabolic pathways due to fermentation can activate secondary metabolic pathways that increase β-Carotene synthesis (Wang et al., 2024). According to Adebo et al. (2022), fermentation reduces the content of anti-nutritional compounds that can inhibit the absorption of β-carotene. Reducing anti-nutrients in feed can increase the absorption of β-carotene in the livestock body. Processing with the fermentation method can also maintain the stability of β-carotene by reducing oxidation during storage so that its content is retained until it is given to livestock. Fermentation using STk-AA1 has been shown to increase the content of β-Carotene in moringa leaf flour.

The content of β-carotene in feed greatly benefits maintaining health and increasing livestock productivity. β-carotene is a source of provitamin A, which plays a role in maintaining health, immunity, and reproductive function in male and female livestock (Pasquariello et al., 2022). β-Carotene compounds can reduce cell damage due to oxidative stress, suppress inflammatory responses, and modulate apoptosis pathways in acute pancreatitis (Gezer et al., 2024). This makes livestock more resistant to disease.

Regarding increasing productivity, β-carotene is essential in feed efficiency, optimizing growth, and producing maximum harvest weight. β-carotene compounds can also help improve meat's color and shelf life and reduce saturated fat levels (Akacha et al., 2023). β-carotene can also enhance egg yolk color and poultry's nutritional content. Fermentation using STk-AA1 can be a solution to increase the levels of β-carotene compounds in moringa leaves.

The measurement results of β-Carotene content, which continued to increase until the 8th day, indicated that a longer fermentation time would increase the β-Carotene content in moringa leaves. Based on the discussion, it can be concluded that processing with the fermentation method using STk-AA1 effectively increases the nutritional content of feed ingredients from local materials, especially moringa leaf flour. However, further research regarding the fermentation time limit is needed to obtain optimal results. In addition, additional research is needed regarding the effects of fermented moringa leaf flour using STk-AA1 on livestock responses.

***Changes in total phenol content of moringa leaf flour fermented using Trichoderma koningiopsis AA1*:** Total phenol is the value of all phenolic compounds contained in a material. Total phenol can act as an antinutrient in animal feed if given excessively. Some types of phenolic compounds have adverse effects on livestock digestion and metabolism. Phenolic compounds such as tannins can inhibit the work of digestive enzymes, thereby reducing the efficiency of

digestion and nutrient absorption (Kraithong et al., 2022). Phenolic compounds are abundant in plants and have antioxidant properties (Feduraev et al., 2022). *Moringa oleifera* leaf flour contains various bioactive compounds, including a phenol compound that acts as a natural antioxidant (Ariani et al., 2023). Phenolic compounds in moringa have antimicrobial effects that can help maintain the health of livestock's digestive tracts and increase feed efficiency (Ullah et al., 2022). However, total phenol levels that are too high will inhibit digestibility due to tannins. To improve the benefits of moringa leaf flour, phenol content can be controlled by a fermentation process so that the antioxidant effect remains maximized without causing adverse impacts on livestock digestion and metabolism.

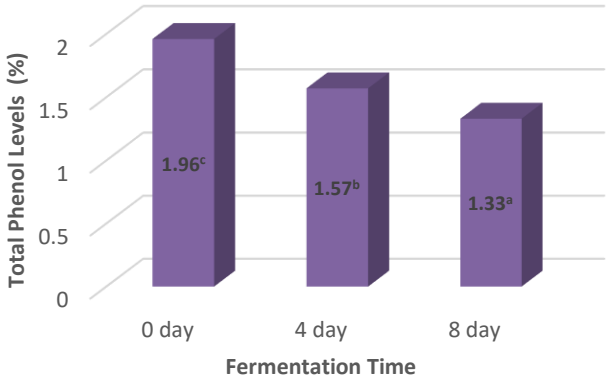

Figure 4. Total phenolic content of moringa leaf flour fermented using *Trichoderma koningiopsis* AA1.

The results of moringa leaf flour fermentation using *Trichoderma koningiopsis* AA1 (STk-AA1) significantly reduced the total phenol content (Table 1). This shows that the fermentation process reduces phenolic compounds in moringa leaf flour. The results of this measurement indicate that fermentation affects the phenolic composition, impacting the functional properties and health benefits of moringa leaf flour. Analysis of variance (ANOVA) showed that the total phenol content decreased significantly ($P < 0.05$). The graphic response of the decrease in total phenol content in moringa leaf flour fermented using STk-AA1 can be seen in Figure 4.

The decrease in total phenol levels during fermentation can be caused by phenolic degradation by STk-AA1, which produces oxidative enzymes in the form of phenol oxidase and peroxidase, which can break down phenolic compounds into other components (Okeke *et al.*, 2024). Microorganism metabolism utilizes phenolic compounds as a carbon and energy source during fermentation (Leonard *et al.*, 2021). Total phenol has benefits as an antioxidant; however, in large amounts, it often acts as an anti-nutrient because it can inhibit the activity of digestive enzymes and reduce the availability of nutrients for livestock (Hafsa et al., 2022). The decrease in total phenol content in fermented moringa leaf flour using STk-AA1 improves the quality of animal feed. Fermentation breaks down complex phenolic compounds into simpler forms (Rocchetti et al., 2022). This makes the protein and energy in the feed more straightforward to digest. The fermentation process can also reduce the toxic effects of phenolic compounds that inhibit the growth of rumen microbes so that the balance of microflora in the livestock digestive system becomes more optimal (Romero et al., 2023).

The decrease in total phenols contributes to increasing the palatability of feed so that livestock production performance can be maximized (Mahfuz et al., 2021). Increased palatability in feed allows livestock to get more nutritional intake to increase their productivity. Reducing the total phenol content through fermentation with STk-AA1 positively impacts growth, milk production, and egg quality. This makes fermented moringa leaf flour a high-quality feed ingredient and helps increase livestock productivity.

The total phenol content in moringa leaf flour fermented using *Trichoderma koningiopsis* AA1 (STk-AA1) continued to decrease significantly ($P < 0.05$) until the 8th day. This indicates that a longer fermentation time will continue to reduce the total phenol content in moringa leaf flour, so further research is needed to determine the optimal time for fermentation of moringa leaf flour using STk-AA1, and in vivo, testing is also needed to determine its effects on livestock.

***Conclusion*:** Fermentation of moringa leaf flour using *Trichoderma koningiopsis* AA1 (STk-AA1) gave a significant effect ($P<0.05$) on increasing the content of flavonoids, vitamin E, and β-carotene and could reduce the total phenol content. The best fermentation time to improve the antioxidant content in moringa leaf flour occurred during 8 days of fermentation.

***CRediT author statement*:** A. M. W. Mulyono, designed, completed the experiments; B. M. Ibrahim prepared the draft; M. Husein, N. H. Qui reviewed and finalized the draft.

***Conflict of interest*:** The authors declare no conflict of interest.

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***SDGs addressed*:** Zero Hunger; Responsible Consumption and Production.

***Policy referred*:** Animal Feed Safety and Quality Policy Sustainable Agriculture and Circular Economy Policies.

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